

Prevalence of brucellosis in ruminants in Bangladesh

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ABSTRACT: The prevalence of brucellosis was determined in the ruminants (buffaloes, cattle, sheep and goats) of five different districts viz. Bagerhat, Bogra, Gaibangha, Mymensingh and Sirajgonj of Bangladesh. A total of 550 sera samples of 105 buffaloes, 188 cattle, 127 goats and 130 sheep were screened by RBT and were further confirmed with I-ELISA. A structured questionnaire was used to collect epidemiological information on the animals. The overall serological prevalence derived from the samples was 2.87% in buffaloes, 2.66% in cattle, 3.15% in goats, and 2.31% in sheep. The prevalence was relatively higher in females than that in males in cattle, goats and sheep but, an insignificantly higher prevalence was observed in males than that in females in the case of buffalo. A significant association was found between abortion or age and occurrence of brucellosis ($P < 0.01$). The results of the study provide (a) a comparison of the prevalence of brucellosis in different livestock species in Bangladesh, (b) constitute baseline data for further study of *Brucella* infections, and (c) are a starting point for the control of brucellosis.

Keywords: brucellosis; ruminants; Bangladesh; seroprevalence

List of abbreviations

ELISA = enzyme-linked immunosorbent assay; I-ELISA = indirect enzyme-linked immunosorbent assay; RBT = Rose-Bengal test; SAT = standard plate agglutination test; TAT = standard tube agglutination test

Brucellosis is a highly contagious and important zoonotic disease caused by different species of the genus *Brucella*, small, Gram negative, non-motile, non spore forming, rod shaped (coccobacilli) bacteria (Baek et al., 2003; Kakoma et al., 2003) that are pathogenic for a wide variety of animals and also for humans (Mathur, 1971). *Brucella* spp. are facultative intracellular parasites causing chronic disease which may persist for the whole life of the affected organism. In animals, brucellosis mainly affects reproduction and fertility, reduces the survival of newborns, and diminishes milk yield. The mortality of adult animals is insignificant (Sewel and Blocklesby, 1990). In human beings, the symptoms of disease are weakness, joint and muscle pain, headache, undulant fever, hepatomegaly, splenomegaly, night sweats and chills, marked asthenia and anorexia (Hugh-Jones, 2000).

In the agro-based economy of Bangladesh livestock contribute 2.73% of the total GDP and 80%

of rural people are directly or indirectly involved with livestock rearing. There are an estimated 23.4 million cattle, 1.86 million buffaloes, 33.5 million goats, 1.1 million sheep being reared in Bangladesh. The importance of brucellosis is not known precisely, but it can have a considerable impact on human and animal health, as well as on socioeconomic factors, as rural income relies largely on livestock breeding and dairy products and people usually live in very close proximity with their livestock. There are a lot of undiagnosed cases of abortion, stillbirth and retained placenta which are thought to be down to brucellosis and these have a significant impact on the development of livestock in Bangladesh (Islam et al., 1983; Rahman et al., 2006; Rahman, 2010).

In Bangladesh, brucellosis was first identified in cattle in 1967 (Mia and Islam, 1967), in buffalo in 1997 (Rahman et al., 1997) and our group along with others reported brucellosis in one or two species of livestock as well as humans (Amin et al.,

2005; Uddin and Rahman, 2007; Nahar and Ahmed, 2009; Rahman et al., 2009, 2010; Ahasan et al., 2010; Muhammad et al., 2010).

Serological tests using the RBT, SAT, TAT, mercaptoethanol test and ELISA are generally used for the detection of *Brucella* infection in livestock. ELISAs have been evaluated for many years for their ability to detect serum antibodies to brucellosis in domestic animals. ELISA for the diagnosis of brucellosis has several advantages when compared with other tests. Firstly, it directly identifies a specific antibody. Secondly, it is more sensitive than other agglutination tests and thus has the potential to detect infected animals. Thirdly, ELISA results provide an epidemiological tool for investigating the infection status of flocks in places where vaccination has never been practiced, like Bangladesh (Rahman, 2005).

To the best of knowledge there is no published comparative report of the sero-prevalence of brucellosis in various livestock species which takes most of the livestock species in Bangladesh into consideration. Therefore, the following study was carried out for the diagnosis of brucellosis in ruminants (cattle, buffaloes, sheep, goats) with these objectives: the detection of brucellosis in various livestock species using RBT as a screening test; the application of I-ELISA to determine the sero-prevalence of brucellosis as a confirmatory test; the epidemiological study of brucellosis in various livestock species in different regions of Bangladesh.

MATERIAL AND METHODS

The study was conducted for a period of 14 months from May 2010 to June 2011 in the Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Experimental design

As serological samples, venous blood samples were randomly and aseptically obtained from sexually mature cattle, buffalo, goats, and sheep of both sexes. A total of 550 serum samples were randomly collected from the Bagherhatt, Bogra, Gaibandha, Mymensingh and Sirajgong regions of Bangladesh; 105 from buffaloes, 188 from cattle, 127 from goats and 130 serum samples from sheep. The study

also recorded required clinical, epidemiological and reproductive information. During sampling a questionnaire based on age, sex, area, pregnancy status, disease history, reproductive problems such as abnormal uterine discharge, abortion and reproductive diseases was filled out.

RBT and I-ELISA

All the blood samples were processed for sera preparation and then subjected to RBT as a screening test in order to identify animals infected with brucellosis using *Brucella abortus* strain 1119-3 (Dae Sung Microbiological lab, South Korea) and the results were confirmed by I-ELISA (Svanova Biotech AB, Uppsala, Sweden). RBT was performed according to the procedure described by the OIE (2008). The test serum samples and Rose-Bengal antigen were kept for one hour at room temperature before the beginning of the test. A result was considered positive when there was any degree of agglutination noticeable and the absence of agglutination was considered as negative. I-ELISA was performed according to the protocol provided by the ELISA kit manufacturer company.

Statistical analysis

The questionnaire-based data was processed by Microsoft Excel and MSTATC and the results were statistically analyzed for interpretation using Chi-square tests (χ^2). Probabilities associated with the observed values of chi-square were determined from relevant tables. Significance was determined at 1% and 5% levels where applicable.

RESULTS

Overall sero-prevalence of brucellosis in different ruminant species

Serum samples were collected from 550 ruminants (105 buffaloes, 188 cattle, 127 goats, and 130 sheep) of five different regions (Mymensingh, Gaibandha, Bogra, Bagherhatt, and Sirajgonj). The numbers of positive reactors by RBT was two out of 105 (1.90%) in buffaloes, four out of 188 (2.13%) in cattle, six out of 127 (4.72%) in the case of goats, and four out of 130 (3.08%) in sheep. The numbers

Table 1. Overall sero-prevalence of brucellosis in different livestock species based on RBT and I-ELISA

Species	Total number of sera tested	Total number of RBT positive reactors (%)	Total number of ELISA positive reactors (%)
Buffaloes	105	2 (1.90)	3 (2.87)
Cattle	188	4 (2.13)	5 (2.66)
Goats	127	6 (4.72)	4 (3.15)
Sheep	130	4 (3.08)	3 (2.31)

of positive reactors by I-ELISA was three out of 105 (2.87%) in buffaloes, five out of 188 (2.66%) in cattle, four out of 127 (3.15%) in goats, and three out of 130 (2.31%) in sheep (Table 1) in the five different regions of Bangladesh.

Age related sero-prevalence of brucellosis in different ruminant species in Bangladesh

Age-wise sero-prevalence of brucellosis is shown in Table 2. In the case of buffaloes, six buffaloes from the 13–24 month age group showed no positive reactions but in the 25–48 month age group, the sero-prevalence was 2.63% (one out of 38 samples) and in the >48 months age group this value was 3.29% (two out of 61). In the case of cattle, six samples from the 13–24 month age group were found to be negative but in the 25–48 months age group, the sero-prevalence was 1.45% (one out of 69 samples) and in cattle in the over 48 month age group the sero-prevalence was 3.54% (four out

of 113). The sero-prevalence of brucellosis in goats of less than 24 months of age was 3.22% (two out of 115). In goats in the age group of over 24 months, the prevalence of brucellosis was 18.18% (two out of 11). In the case of sheep, in 110 sheep of less than 24 months of age, the prevalence of brucellosis was 0.00% but in the age group of over 24 months, the prevalence of brucellosis was 15.0% (three out of 20) in the five different regions of Bangladesh.

Sex-related sero-prevalence of brucellosis

Sex-related sero-prevalence of brucellosis is shown in Table 3. A relatively higher prevalence was found in females than in males in cattle, goat and sheep whereas a higher prevalence of brucellosis was found in males than in females in the case of buffaloes.

In the case of buffaloes 14 males and 91 females were tested and the prevalence of brucellosis was 7.14% in the case of males whereas a 2.02% preva-

Table 2. Age related sero-prevalence of brucellosis based on RBT and I-ELISA in different livestock species

Species	Age of animals (months)	Number of sera tested	Number of positive reactors by RBT (%)	Number of positive reactors by I-ELISA (%)
Buffaloes	13–24	6	0	0
	25–48	38	1 (2.63)	1 (2.63)
	> 48	61	3 (4.92)	2 (3.29)
Cattle	13–24	6	0	0
	25–48	69	1 (1.45)	1 (1.45)
	> 48	113	6 (5.31)	4 (3.54)
Goats	< 24	115	2 (3.22)	2 (3.22)
	> 24	11	2 (18.18)	2 (18.18)
Sheep	< 24	110	0	0
	> 24	20	3 (15.0)	3 (15.0)*

*significant at 1% level of probability ($P < 0.01$)

Table 3. Sex related sero-prevalence of brucellosis based on RBT and I-ELISA in different livestock species

Species	Sex of animals	Number of sera tested	Number of positive reactors by RBT (%)	Number of positive reactors by I-ELISA (%)
Buffaloes	male	14	1 (7.14)	1 (7.14)
	female	91	3 (3.30)	2 (2.02)
Cattle	male	25	0	0
	female	163	7 (4.29)	5 (3.07)
Goats	male	28	0	0
	female	99	4 (4.04)	4 (4.04)*
Sheep	male	15	0	0
	female	115	3 (2.61)	3 (2.61)*

*significant at 1% level of probability ($P < 0.01$)

lence was found in females. In cattle 25 sera samples from male were tested and none reacted positively with RBT and I-ELISA. In females the prevalence was 3.07%. In goats, 3.57% of male sera were found to be positive reactors. The average sero-prevalence was 4.04% in female goats. In sheep, a 0.0% prevalence was found with every test performed in males but in females the average prevalence was 2.61% with I-ELISA.

The distribution of brucellosis with regard to abortion history

The distribution of brucellosis with respect to abortion history in the tested animals is presented in Table 4. Among 468 female animals, five buffa-

loes, seven cattle, three goats, and two sheep had a record of a previous abortion. The sero-prevalence of brucellosis in the aborted animals was 60.0% in buffaloes, 57.14% in cattle, 66.67% in goats and 50.0% in sheep. There existed a significant association in buffaloes, cattle, goats and sheep between abortion and the prevalence of brucellosis ($P < 0.01$). Based on this, it can be concluded that the prevalence of brucellosis was significantly higher in animals with a previous abortion record than in animals with no abortion record.

DISCUSSION

Brucellosis is an important zoonosis and serological surveillance is essential to its control

Table 4. Prevalence of brucellosis in animals of different livestock species with a previous abortion based on RBT and I-ELISA

Species	Previous abortion record (yes/no)	Number of sera tested	Number of positive reactors by RBT (%)	Number of positive reactors by I-ELISA (%)
Buffaloes	yes	5	3 (60.0)	3 (60.0)*
	no	86	1 (1.16)	0
Cattle	yes	7	5 (71.43)	4 (57.14)*
	no	156	2 (1.28)	1 (0.64)
Goats	yes	3	2 (66.67)	2 (66.67)*
	no	96	2 (2.08)	2 (2.08)
Sheep	yes	2	1 (50.0)	1 (50.0)*
	no	113	2 (1.77)	2 (1.77)

*significant at 1% level of probability ($P < 0.01$)

(Erdenebaatar et al., 2004). Although many countries have eradication programs for controlling brucellosis, economic losses can be heavy due to abortion and infertility and subsequent culling so herds should be monitored for the presence of infection. Despite eradication programs, including vaccination, testing and slaughter, brucellosis remains a major zoonosis worldwide (Matyas and Fujikura, 1984; WHO, 1986; Baek et al., 2003; Kakoma et al., 2003) and the disease has remained prevalent in many areas in the world. Each year half a million cases of brucellosis are reported worldwide but according to WHO, these numbers are greatly underestimated. In recent years, many countries have eradicated brucellosis from their herds, and many other countries have significantly reduced the prevalence of the infection among their livestock populations. Even so, brucellosis is distributed throughout the world wherever livestock are being raised. Likewise, in many less developed countries and in developing countries brucellosis continues to cause major losses in livestock and poses a serious threat to people (Crawford et al., 1990). The distribution of the disease is geographically limited, but it nevertheless remains a major problem in Western and southern Asia, parts of Africa and Latin America (Amato, 1995).

The objectives of the study were to make a comparative study of the sero-prevalence of brucellosis in different ruminant species in Bangladesh and to improve the understanding of the epidemiology of *Brucella* in buffaloes, cattle, goats and sheep and to provide information for disease control in ruminants in Bangladesh. Sero-positivity was considered to be due to natural infection because vaccination has never been practiced in Bangladesh. The results showed that brucellosis in buffaloes, cattle and goats was widespread and occurred at much higher prevalences than in sheep. There are no comparative studies on the prevalence of brucellosis in different livestock in Bangladesh. The present study revealed that in the studied area the overall sero-prevalence of brucellosis in buffaloes was 2.87% by I-ELISA. In a previous study on buffaloes (El-Bassiony et al., 2007), 50 milk and whey samples were examined and a prevalence of 0.0% was found but a brucellosis prevalence of 2.0% has been reported in buffalo (Zikusoka et al., 2005). Further, Pushpa and Kumari (2005) reported a prevalence 5.58% using RBT which is higher than the current study.

The current investigation revealed that the overall sero-prevalence of brucellosis in cattle was 2.13%

by I-ELISA in five regions. This finding is in agreement with Rahman et al. (2006) who reported an animal-level sero-prevalence of brucellosis in cattle of 2.4–8.4% with a herd incidence in cattle of 62.5%. In the case of goats the prevalence was found to be 3.15% by I-ELISA which is higher than the 1.98% reported by Ahasan et al. (2010) and the 2.33% reported by Uddin and Rahman (2007), but lower than Rahman et al. (1988) who reported 14.57% positive cases of brucellosis in caprines in different regions of Bangladesh where there exists ample opportunities for the intermixing of species, grazing lands and composite small holdings of livestock maintained by nearly 80% of the rural population. Among all the livestock species in Bangladesh, goats were found to be most infected with brucellosis. The prevalence and severity of disease may vary with the breed, geographic location, type of diagnostic test, husbandry and environmental factors (Amin et al., 2005). Therefore, the gap in these sero-prevalences will be mainly down to the selected area (or farm) and to the applied diagnostic methods. The present study included mostly indigenous animals from five different regions with different husbandry and environmental situations. There is also a difference in the diagnostic methods used; the above authors mostly employed RBT whereas we used I-ELISA as a confirmatory test. RBT generally has higher sensitivity and lower specificity compared to I-ELISA. Thus, when using screening tests such as RBT, a high prevalence can be determined. Other contributory factors to variation include contact with wild animals and housed dogs.

In our study there was a significant association between age and the prevalence of brucellosis. With respect to the age-related sero-prevalence in three age groups of cattle and buffaloes, the highest prevalence of brucellosis was found in buffaloes and cattle of more than 48 months of, which was 3.29% and 3.54% by I-ELISA, respectively. There was a significant association between age group and the prevalence of brucellosis in buffaloes and cattle because the majority of positive reactors were found in buffaloes and cattle above 48 months of age. This finding correlates with the observation of Chantal and Thomas (1977), who found a high prevalence of brucellosis (8.7%) in cattle aged five to 10 years. Similar observations were also recorded by other investigators (Botha and Williamson, 1989; Muranalini and Ramasastry, 1999). It is possible that the higher prevalence of brucellosis among older cows may be related to their advanced age,

as the organism may remain latent or chronic for an unspecified period before manifesting as clinical disease. Alternatively, the aged animals have a greater chance of becoming infected and of coming into contact with other animals.

In this study it was revealed that the prevalence of brucellosis was relatively higher in females than in the males of cattle, goats and sheep but in buffaloes, an insignificantly higher prevalence was observed in males than in females. Lavsén et al. (1988) found a higher prevalence of brucellosis among females in Victoria, Canada. The higher rate of infection in females will be due to infection within the female reproductive tract providing a potential reservoir for the organism to propagate.

We observed a positive association between previous abortion record and the prevalence of brucellosis in buffaloes, cattle, goats and sheep. The prevalence of brucellosis in buffaloes, cattle, goats and sheep with a previous abortion record was 60.0%, 57.14%, 66.67%, 50.0% and 0.0%, respectively. Ibrahim and Habiballa (1975) reported a prevalence of brucellosis of 14.2% in cows that had previously aborted. Other researchers have reported similar findings (Sandoval et al., 1979; Shaw, 1986; Barman et al., 1989; Sandhu et al., 2001). Among all of the reproductive disorders that were investigated, a history of previous abortion was associated with the highest prevalence of brucellosis.

The results of the study provide a comparison of the prevalence of brucellosis in different livestock species in Bangladesh and constitute baseline data for the further study of *Brucella* infections as well as a starting point for the control of brucellosis.

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